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I, DAVID DANIEL CLARKE , ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed are true copies of the Provisional specification and drawing(s) as filed on 2 March 1995 in connection with Application No. PN 1457 for a patent by THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH and AMRAD CORPORATION LIMITED filed on 2 March 1995.

I further certify that the annexed documents are not, as yet, open to public inspection.

COMMONWEALTH OF AUSTRALIA
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WITNESS my hand this Twenty-eighth
day of February 1996

DAVID DANIEL CLARKE
ASSISTANT DIRECTOR PATENT SERVICES

Regulation 3.2

The Council of the Queensland Institute
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A U S T R A L I A
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PROVISIONAL SPECIFICATION
for the invention entitled:

"A Novel Growth Factor and a Genetic Sequence Encoding Same"

The invention is described in the following statement:

A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME

The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Vascular endothelial growth factor (hereinafter referred to as "VEGF"), also known as vasoactive permeability factor, is a secreted, covalently linked homodimeric glycoprotein that specifically activates endothelial tissues (Senger *et al.*, 1993). A range of functions have been attributed to VEGF such as its involvement in normal angiogensis including formation of the corpus luteum (Yan *et al.*, 1993) and placental development (Sharkey *et al.*, 1993), regulation of vascular permeability (Senger *et al.*, 1993), inflammatory angiogenesis (Sunderkotter *et al.*, 1994) and autotransplantation (Dissen *et al.*, 1994) and human diseases such as tumour promoting angiogenesis (Folkman & Shing, 1992), rheumatoid arthritis (Koch *et al.*, 1994) and diabetes related retinopathy (Folkman & Shing, 1992).

VEGF is, therefore, an important molecule making it a potentially valuable target for research into therapeutics, prophylactics and diagnostic agents based on VEGF or its activities. There is also a need to identify homologues or otherwise related molecules for use as an alternative to VEGF or in conjunction with VEGF.

In work leading up to the present invention, the inventors sought the multiple endocrine neoplasia type I susceptibility gene (MEN1). Surprisingly, the inventors discovered that a genetic sequence excluded as a candidate for the MEN1 gene was nevertheless a new growth factor having some similarity to VEGF.

Accordingly, one aspect of the present invention comprises a biologically isolated proteinaceous molecule comprising a sequence of amino acids which:

(i) is at least about 30% similar to the amino acid sequence set forth in SEQ ID NO:1; and

(ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:1.

Another aspect of the present invention provides a biologically isolated proteinaceous molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 30% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:1;

(ii) exhibits at least one property in common with VEGF.

A related aspect of the present invention contemplates a biologically isolated proteinaceous molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 30% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:1;

(ii) exhibits at least one of the following properties:

(a) ability to induce proliferation of vascular endothelial cells;

(b) ability to interact with *flt*-1/*flk*-1 family of receptors;

(c) ability to induce cell migration, cell survival and/or an increase in

intracellular levels of alkaline phosphatase.

By "biologically isolated" is meant that the molecule has undergone at least one step of purification from a biological source. Preferably, the molecule is also biologically pure meaning that a composition comprises at least about 20%, more preferably at least about 40%, still more preferably at least about 65%, even still more preferably at least about 80-90% or greater of the molecule as determined by weight, activity or other convenient means, relative to other compounds in the composition. Most preferably, the molecule is sequencably pure.

Another preferred aspect of the present invention provides the molecule in recombinant form.

According to this aspect of the present invention, there is provided a recombinant molecule comprising a sequence of amino acids which:

(i) is at least about 30% similar to the amino acid sequence set forth in SEQ ID NO:1; and

(ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:1.

A related aspect of the present invention is directed to a recombinant molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 30% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:1;

(ii) exhibits at least one property in common with VEGF.

A further related aspect of the present invention contemplates a recombinant molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 30% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:1;

(ii) exhibits at least one of the following properties:

(a) ability to induce proliferation of vascular endothelial cells;

(b) ability to interact with *flt*-1/*flk*-1 family of receptors;

(c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

The amino acid sequence set forth in SEQ ID NO:1 corresponds to human VEGF (referred to herein as "$VEGF_{165}$"). Accordingly, the molecule of the present invention is VEGF-like or is a homologue of VEGF but comprises an amino acid sequence which is similar but non-identical to the amino sequence of VEGF. Although the present invention is exemplified using a human VEGF-like molecule, this is done with the understanding that the instant invention contemplates the homologous molecule and encoding sequence from other mammals such as livestock animals (e.g. sheep, pigs, horses and cows), companion animals (e.g. dogs and cats) and laboratory test animals (e.g. mice, rats, rabbits and guinea pigs) as well as non-mammals such as birds (e.g. poultry birds), fish and reptiles. In a most preferred embodiment, the VEGF-like molecule is of human origin and encoded by a gene located at chromosome 11q13. The present invention extends, therefore, to the genomic sequence or part thereof encoding the subject VEGF-like molecule.

In a particularly preferred embodiment, the VEGF-like molecule of the present invention comprises a sequence of amino acids as set forth in SEQ ID NO:4 or is a part, fragment, derivative or analogue thereof. The amino acid sequence set forth in SEQ ID NO:4 is also referred to herein as "$SOM175_{long}$". In another embodiment, the VEGF-like molecule of the present invention comprises a sequence of amino acids as set forth in SEQ ID NO:3 or is a part, fragment, derivative or analogue thereof. The amino acid sequence set forth in SEQ ID NO:3 is also referred to herein as "$SOM175_{short}$". The two amino acid sequences stem from a potential frame shift caused by insertion of an additional nucleotide between positions 500 and 532 or by deletion of two nucleotides between positions 394 and 532 in the nucleotide sequence set forth in SEQ ID NO:2.

Another embodiment provides a recombinant molecule having the following characteristics:

(i) an amino acid sequence substantially as set forth in SEQ ID NO:3 or SEQ ID NO:4 or having at least about 30% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:1;

(ii) exhibits at least one biological property in common with VEGF.

Such properties of VEGF include at least one of:

(a) ability to induce proliferation of vascular endothelial cells;

(b) an ability to interact with *flt*-1/*flk*-1 family of receptors;

(c) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

In accordance with the present invention, a preferred similarity is at least about 40%, more preferably at least about 50% and even more preferably at least about 65% similarity.

Still a further aspect of the present invention contemplates a peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 or a chemical equivalent thereof. The biologically isolated or recombinant molecule of the present invention may be naturally glycosylated or may comprise an altered glycosylation pattern depending on the cells from which it is isolated or synthesised. For example, if produced by recombinant means in prokaryotic organisms, the molecule would be non-glycosylated. The molecule may be a full length, naturally occurring form or may be a truncated or otherwise derivatised form.

Yet another aspect of the present invention is directed to a nucleic acid molecule encoding the VEGF-like molecule herein described. More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:2 or having at least 30% similarity thereto or being capable of hybridising under low stringency conditions to a reverse complement

of the nucleotide sequence as set forth in SEQ ID NO:2 provided that the nucleic acid sequence having at least 30% similarity but at least 5% dissimilarity to the amino acid sequence as set forth in SEQ ID NO:1. The nucleotide sequence set forth in SEQ ID NO:2 is also referred to herein as "SOM175".

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al* (1989) at pages 9.47-9.51 which is herein incorporated by reference where the washing steps disclosed are considered high stringency. A low stringency is defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at ≥ 45°C for 2-3 hours or high stringent conditions considered herein to be 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

The present invention further contemplates a nucleic acid molecule which encodes a VEGF-like molecule as hereinbefore described having at least 30% sequence homology to SEQ ID NO:5. Preferably, the level of homology is at least about 40%, more preferably at least about 60% and even more preferably at least about 70%.

The VEGF-like molecule of the present invention will be useful in the development of a range of therapeutic and/or diagnostic applications alone or in combination with other molecules such as VEGF. The present invention extends, therefore, to pharmaceutical compositions comprising the VEGF-like molecule or parts, fragments, derivatives, homologues or analogues thereof together with one or more pharmaceutically acceptable carriers and/or diluents. Furthermore, the present invention extends to vectors comprising the nucleic acid sequence set forth in SEQ ID NO:2 or having at least about 30%, more preferably about 50% and even more preferably about 70% or above similarity thereto and host cells comprising same. In addition, the present invention extends to ribozymes and antisense molecules based on SEQ ID NO:2 as well as neutralizing antibodies to the VEGF-like molecule. Such molecules may be useful in ameliorating the effects of, for example, over expression of VEGF-like genes leading

to angiogenesis or vascularization of tumours.

The present invention also contemplates antibodies to the VEGF-like molecule or nucleic acid probes to a gene encoding the VEGF-like molecule which are useful as diagnostic agents.

The present invention is further described by reference to the following non-limiting Figures and/or Examples.

In the Figures:

Figure 1A Amino acid sequence of $VEGF_{165}$ (SEQ ID NO:1)

Figure 1B Nucleotide sequence of VEGF (SEQ ID NO:5)

Figure 2 Nucleotide sequence of SOM175 (SEQ ID NO:2)

Figure 3 Results of BLAST search with SOM175 protein sequence

Figure 4 BESTFIT alignment of VEGF cDNA and SOM175 cDNA

Figure 5A BESTFIT analysis of $SOM175_{short}$ amino acid sequence (SEQ ID NO:3) with $VEGF_{165}$ amino acid sequence

Figure 5B BESTFIT analysis of $SOM175_{long}$ amino acid sequence (SEQ ID NO:4) with $VEGF_{165}$ amino acid sequence

Figure 6 Representation of an alignment of $VEGF_{165}$ protein with the two embodiments of SOM175.

SUMMARY OF SEQUENCE IDENTITY NUMBERS

SEQ ID NO:1	Amino acid sequence of $VEGF_{165}$
SEQ ID NO:2	Nucleotide sequence of SOM175 (VEGF-like molecules)
SEQ ID NO:3	Amino acid sequence of $SOM175_{short}$
SEQ ID NO:4	Amino acid sequence of $SOM175_{long}$.
SEQ ID NO:5	Nucleotide sequence of $VEGF\text{-}_{165}$cDNA.

EXAMPLE 1

cDNA was isolated by screening a human foetal brain library (LambdazapII, Stratagene) with the cosmid D11S750 (Larsson *et al.*, 1992). The insert was excised *in vivo* and the plasmid clone SOM175 with a 1.1kb insert was obtained. This clone was restriction mapped, subcloned and sequenced using fluorescently labelled cycle sequencing and was run on a 373A automated sequencer (Applied Biosystems) using methods described by the manufacturer.

EXAMPLE 2

DNA SEQUENCE ANALYSIS

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in Figure 2. This sequence was screened for open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed

(see Figure 2). The amino acid sequence is shown in Figure 6. There appears to be little 5′ untranslated sequences (2bp). The 3′ untranslated region appears to be complete as it includes a poly-adenylation signal and poly-A tail (see Figure 2).

Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see Figure 3). The amount of homology between SOM175 and human $VEGF_{165}$ was determined using the BESTFIT program (GCG, University of Wisconsin; see Figures 4 and 5). Nucleotide homology was estimated at 69.7% and protein homology was estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams *et al.*, 1993).

These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47, Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in Figure 6. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has unique properties of its own.

EXAMPLE 3

The percentage similarity and divergence between VEGF family and SOM175 family (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 1 and 2. The human VEGF family consists of a range of VEGF molecules of varying size, known as $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$ (Tischer *et al.*, 1991). The two reading frames for SOM175 (SEQ ID NO:2) are represented as $SOM175_{long}$ (SEQ ID NO:4) and $SOM175_{short}$ (SEQ ID NO:3).

Table 1

Percent Similarity

	$VEGF_{121}$	$VEGF_{165}$	$VEGF_{189}$	$VEGF_{206}$	$SOM175_{long}$	$SOM175_{short}$
$VEGF_{121}$	***	97.3	98.0	98.0	34.0	34.0
$VEGF_{165}$		***	97.9	97.9	34.6	27.2
$VEGF_{189}$			***	98.6	32.6	25.6
$VEGF_{206}$				***	32.0	26.1
$SOM175_{long}$					***	85.0
$SOM175_{short}$						***

Percent Divergence

	$VEGF_{121}$	$VEGF_{165}$	$VEGF_{189}$	$VEGF_{206}$	$SOM175_{short}$	$SOM175_{long}$
$VEGF_{121}$	***	0.0	0.0	0.0	57.4	55.1
$VEGF_{165}$		***	0.0	0.5	63.2	54.8
$VEGF_{189}$			***	0.0	67.4	59.7
$VEGF_{206}$				***	68.1	61.0
$SOM175_{short}$					***	14.5
$SOM175_{long}$						***

EXAMPLE 4

BIOASSAYS TO DETERMINE THE FUNCTION OF SOM175

Assays are conducted to evaluate whether SOM175 has similar activities to VEGF on endothelial cell function, angiogenesis and wound healing. Other assays are performed based on the results of receptor binding distribution studies.

Assays of endothelial cell function

Endothelial cell proliferation. Endothelial cell growth assays as described in Ferrara & Henzel (1989) and in Gospodarowicz *et al* (1989).

Vascular permeability assay. This assay, which utilises the Miles test in guinea pigs, will be performed as described in Miles & Miles (1952).

Cell adhesion assay. The influence of SOM175 on adhesion of polymorphs to endothelial cells is analysed.

Chemotaxis. This is performed using the standard Boyden chamber chemotaxis assay.

Plasminogen activator assay. Endothelial cells are tested for plasminogen activator and plasminogen activator inhibitor production upon addition of SOM175 (Pepper *et al* (1991)).

Endothelial cell migration assay. The ability of SOM175 to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano *et al* (1986).

Angiogenesis Assay

SOM175 induction of an angiogenic response in chick chorioallantoic membrane is evaluated as described in Leung *et al* (1989).

Possible neurotrophic actions of SOM175 are assessed using the following assays:

Neurite outgrowth assay and gene induction (PC12 cells)

PC12 cells (a phaeochromocytoma cell line) respond to NGF and other neurotrophic factors by developing the characteristics of sympathetic neurons, including the induction of early and late genes and the extension of neurites. These cells are exposed to SOM175 and their response monitored (Drinkwater *et al* (1991); and Drinkwater *et al* (1993)).

Cultured neurons from the Peripheral Nervous System (PNS)

Primary cultures of the following PNS neurons are exposed to SOM175 and monitored for any response:

- sensory neurons from neural crest and dorsal root ganglia
- sympathetic neurons from sympathetic chain ganglia
- placode derived sensory neurons from nodose ganglia
- motoneurons from spinal cord

The assays are described in Suter *et al* (1992) and in Marinou *et al* (1992).

Where an *in vitro* response is observed, *in vivo* assays for properties such as uptake and retrograde transport are performed as described in Hendry *et al* (1992).

Nerve regeneration (PNS)

Where neurotrophic effects of SOM175 are observed, its possible role in the regeneration of axotomised sensory neurons, sympathetic neurons and motoneurons is analysed by the methods of Otto *et al* (1989); Yip *et al* (1984) and Hendry *et al* (1976).

Actions of SOM175 on CNS neurons

The ability of SOM175 to promote survival of central nervous system neurons is analysed as described in Hagg *et al* (1992); Williams *et al* (1986); Hefti (1986) and Kromer (1987).

Wound Healing

The ability of SOM175 to support wound healing are tested in the most clinically relevant model available, as described in Schilling *et al* (1959) and utilised by Hunt *et*

al (1967).

The Haemopoietic System

A variety of *in vitro* and *in vivo* assays on specific cell populations of the haemopoietic system are available and are outlined below:

Stem Cells

Murine

A variety of novel *in vitro* murine stem cell assays have been developed using FACS-purified cells:

(a) Repopulating Stem Cells

These are cells capable of repopulating the bone marrow of lethally irradiated mice, and have the Lin^-, Rh^{hi}, Ly-6A/E^+, c-kit^+ phenotype. The test substance is tested on these cells either alone, or by co-incubation with multiple factors, followed by measurement of cellular proliferation by ^3H thymidine incorporation.

(b) Late Stage Stem Cells

These are cells that have comparatively little bone marrow repopulating ability but can generate D13 CFU-S. These cells have the Lin^-, Rh^{hi}, Ly-6A/E^+, c-kit^+ phenotype. The test substance is incubated with these cells for a period of time, injected into lethally irradiated recipients, and the number of D13 spleen colonies enumerated.

(c) Progenitor-Enriched Cells

These are cells that respond *in vitro* to single growth factors, and have the Lin^-, Rh^{hi}, Ly-6A/E^+, c-kit^+ phenotype. This assay will show if SOM175 can act directly on haemopoietic progenitor cells. The test substance is incubated with these cells in agar cultures, and the number of colonies enumerated after 7-14 days.

Atherosclerosis

Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change in their phenotype from a contractile to a synthetic state. Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic

modulations of smooth muscle cell. An *in vitro* assay that measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment is used to assess the effect of SOM175 on smooth muscle cells. The system uses a modified Rose chamber in which different cell types are seeded onto opposite coverslips.

Effects of SOM175 on bone

The ability of SOM175 to regulate proliferation of osteoblasts is assayed as described in Lowe *et al* (1991). Any effects on bone resorption are assayed as described in Lowe *et al* (1991). Effects on osteoblast migration and changes in intracellular molecules (e.g. cAMP accumulation, alkaline phosphatase levels) are analysed as described in Midy *et al* (1994).

Effects on skeletal muscle cells

Effects of SOM175 on proliferation of myoblasts and development of myotubes can be determined as described by Ewton *et al* (1980) and by Gospodarowicz *et al* (1976).

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH and AMRAD CORPORATION LIMITED

(ii) TITLE OF INVENTION: A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME

(iii) NUMBER OF SEQUENCES: 5

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(A) APPLICATION NUMBER: AU PROVISIONAL
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(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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1               5                  10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
    130                 135                 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145                 150                 155                 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
                165                 170                 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
            180                 185                 190
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1094 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCATGAGCCC TCTGCTCCGC CGCCTGCTGC TCGCCGCACT CCTGCAGCTG GCCCCCGCCC 60

AGGCCCCTGT CTCCCAGCCT GATGCCCCTG GCCACCAGAG GAAAGTGGTG TCATGGATAG 120

ATGTGTATAC TCGCGCTACC TGCCAGCCCC GGGAGGTGGT GGTGCCCTTG ACTGTGGAGC 180

TCATGGGCAC CGTGGCCAAA CAGCTGGTGC CCAGCTGCGT GACTGTGCAG CGCTGTGGTG 240

GCTGCTGCCC TGACGATGGC CTGGAGTGTG TGCCCACTGG GCAGCACCAA GTCCGGATGC 300

AGATCCTCAT GATCCGGTAC CCGAGCAGTC AGCTGGGGGA GATGTCCCTG GAAGAACACA 360

GCCAGTGTGA ATGCAGACCT AAAAAAAAGG ACAGTGCTGT GAAGCCAGAC AGGGCTGCCA 420

CTCCCCACCA CCGTCCCCAG CCCCGTTCTG TTCCGGGCTG GGACTCTGCC CCCGGAGCAC 480

CCTCCCCAGC TGACATCACC CATCCCACTC CAGCCCCAGG CCCCTCTGCC CACGCTGCAC 540

CCAGCACCAC CAGCGCCCTG ACCCCCGGAC CTGCCGCTGC CGCTGCCGAC GCCGCAGCTT 600

CCTCCGTTGC CAAGGGCGGG GCTTAGAGCT CAACCCAGAC ACCTGCAGGT GCCGGAAGCT 660

GCGAAGGTGA CACATGGCTT TTCAGACTCA GCAGGGTGAC TTGCCTCAGA GGCTATATCC 720

CAGTGGGGGA ACAAAGGGGA GCCTGGTAAA AAACAGCCAA GCCCCCAAGA CCTCAGCCCA 780

GGCAGAAGCT GCTCTAGGAC CTGGGCCTCT CAGAGGGCTC TTCTGCCATC CCTTGTCTCC 840

CTGAGGCCAT CATCAAACAG GACAGAGTTG GAAGAGGAGA CTGGGAGGCA GCAAGAGGGG 900

TCACATACCA GCTCAGGGGA GAATGGAGTA CTGTCTCAGT TTCTAACCAC TCTGTGCAAG 960

TAAGCATCTT ACAACTGGCT CTTCCTCCCC TCACTAAGAA GACCCAAACC TCTGCATAAT 1020

GGGATTTGGG CTTTGGTACA AGAACTGTGA CCCCCAACCC TGATAAAAGA GATGGAAGGA 1080

AAAAAAAAAA AAAA 1094

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1               5                  10                  15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125

Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg
    130                 135                 140

Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro
145                 150                 155                 160

Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala
                165                 170                 175

His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala
            180                 185                 190

Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 222 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1               5                  10                  15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
            20                  25                  30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125

Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg
    130                 135                 140

Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro
145                 150                 155                 160

Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Leu Cys
                165                 170                 175

Pro Arg Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg
            180                 185                 190

Cys Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu
        195                 200                 205

Glu Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 649 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCGGGCCTCC GAAACCATGA ACTTTCTGCT GTCTTGGGTG CATTGGAGCC TTGCCTTGCT 60

GCTCTACCTC CACCATGCCA AGTGGTCCCA GGCTGCACCC ATGGCAGAAG GAGGAGGGCA 120

GAATCATCAC GAAGTGGTGA AGTTCATGGA TGTCTATCAG CGCAGCTACT GCCATCCAAT 180

CGAGACCCTG GTGGACATCT TCCAGGAGTA CCCTGATGAG ATCGAGTACA TCTTCAAGCC 240

ATCCTGTGTG CCCCTGATGC GATGCGGGGG CTGCTGCAAT GACGAGGGCC TGGAGTGTGT 300

GCCCACTGAG GAGTCCAACA TCACCATGCA GATTATGCGG ATCAAACCTC ACCAAGGCCA 360

GCACATAGGA GAGATGAGCT TCCTACAGCA CAACAAATGT GAATGCAGAC CAAAGAAAGA 420

TAGAGCAAGA CAAGAAAATC CCTGTGGGCC TTGCTCAGAG CGGAGAAAGC ATTTGTTTGT 480

ACAAGATCCG CAGACGTGTA AATGTTCCTG CAAAAACACA GACTCGCGTT GCAAGGCGAG 540

GCAGCTTGAG TTAAACGAAC GTACTTGCAG ATGTGACAAG CCGAGGCGGT GAGCCGGGCA 600

GGAGGAAGGA GCCTCCCTCA GCGTTTCGGG AACCAGATCT CTCACCAGG 649

DATED this 2nd day of March, 1995

THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH
and AMRAD CORPORATION LIMITED
By Its Patent Attorneys
DAVIES COLLISON CAVE

FIGURE 1A

[SEQ ID NO:1]

MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKF
MDVYQRSYCHPIETLVDIFQEYP DEIEYIFKPSCVPLMRCGGCCNDE
GLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDRA
RQENPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERT
CRCDKPRR

FIGURE 1B

[SEQ ID NO:5]

TCGGGCCTCCGAAACCATGAACTTTCTGCTGTCTTGGGTGCATTG
GAGCCTTGCCTTGCTGCTCTA CCTCCACCATGCCAAGTGGTCCCA
GGCTGCACCCATGGCAGAAGGAGGAGGGCAGAATCATCACGAAG
TGGTGAAGTTCATGGATGTCTATCAGCGCAGCTACTGCCATCCAA
TCGAGACCCTGGTGGACATCTTCCAGGAGTACCCTGATGAGATCG
AGTACATCTTCAAGCCATCCTGTGTGCCCCTGATGCGATGCGGGG
GCTGCTGCAATGACGAGGGCCTGGAGTGTGTGCCCACTGAGGAG
TCCAACATCACCATGCAGATTATGCGGATCAAACCTCACCAAGGC
CAGCACATAGGAGAGATGAGCTTCCTACAGCACAACAAATGTGA
ATGCAGACCAAAGAAAGATAGAGCAAGACAAGAAAATCCCTGTG
GGCCTTGCTCAGAGCGGAGAAAGCATTTGTTTGTACAAGATCCGC
AGACGTGTAAATGTTCCTGCAAAAACACAGACTCGCGTTGCAAG
GCGAGGCAGCTTGAGTTAAACGAACGTACTTGCAGATGTGACAA
GCCGAGGCGGTGAGCCGGGCAGGAGGAAGGAGCCTCCCTCAGCG
TTTCGGGAACCAGATCTCTCACCAGG

FIGURE 2

[SEQ ID NO:2]

CCATGAGCCCTCTGCTCCGCCGCCTGCTGCTCGCCGCACTCCTGCA
GCTGGCCCCCGCCCAGGCCCCTGTCTCCCAGCCTGATGCCCCTGG
CCACCAGAGGAAAGTGGTGTCATGGATAGATGTGTATACTCGCG
CTACCTGCCAGCCCCGGGAGGTGGTGGTGCCCTTGACTGTGGAGC
TCATGGGCACCGTGGCCAAACAGCTGGTGCCCAGCTGCGTGACTG
TGCAGCGCTGTGGTGGCTGCTGCCCTGACGATGGCCTGGAGTGTG
TGCCCACTGGGCAGCACCAAGTCCGGATGCAGATCCTCATGATCC
GGTACCCGAGCAGTCAGCTGGGGGAGATGTCCCTGGAAGAACAC
AGCCAGTGTGAATGCAGACCTAAAAAAAAAGGACAGTGCTGTGAA
GCCAGACAGGGCTGCCACTCCCCACCACCGTCCCCAGCCCCGTTC
TGTTCCGGGCTGGGACTCTGCCCCCGGAGCACCCTCCCCAGCTGA
CATCACCCATCCCACTCCAGCCCCAGGCCCCTCTGCCCACGCTGC
ACCCAGCACCACCAGCGCCCTGACCCCCGGACCTGCCGCTGCCGC
TGCCGACGCCGCAGCTTCCTCCGTTGCCAAGGGCGGGGCTTAGAG
CTCAACCCAGACACCTGCAGGTGCCGGAAGCTGCGAAGGTGACA
CATGGCTTTTCAGACTCAGCAGGGTGACTTGCCTCAGAGGCTATA
TCCCAGTGGGGGAACAAAGGGGAGCCTGGTAAAAAACAGCCAAG
CCCCCAAGACCTCAGCCCAGGCAGAAGCTGCTCTAGGACCTGGGC
CTCTCAGAGGGCTCTTCTGCCATCCCTTGTCTCCCTGAGGCCATCA
TCAAACAGGACAGAGTTGGAAGAGGAGACTGGGAGGCAGCAAG
AGGGGTCACATACCAGCTCAGGGGAGAATGGAGTACTGTCTCAG
TTTCTAACCACTCTGTGCAAGTAAGCATCTTACAACTGGCTCTTCC
TCCCCTCACTAAGAAGACCCAAACCTCTGCATAATGGGATTTGGG
CTTTGGTACAAGAACTGTGACCCCCAACCCTGATAAAAGAGATGG
AAGGAAAAAAAAAAAAAAA

FIGURE 3

```
>VEGF_HUMAN VEGF_HUMAN  VASCULAR ENDOTHELIAL GROWTH FACTOR PRECURSOR (VEGF)
            (VASCULAR 215 AA.
            Length = 215

 Score = 181 (92.4 bits), Expect = 6.4e-20, P = 6.4e-20
 Identities = 33/75 (44%), Positives = 48/75 (64%)

Query:    31 HQRKVVSWIDVYTRATCQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECV 90
             +++ VV  +DVY R+ C+P E +V +  E     +      PSCV + RCGGCC D+GLECV
Sbjct:    36 NHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECV 95

Query:    91 PTGQHQVRMQILMIR 105
             PT +  + MQI+ I+
Sbjct:    96 PTEESNITMQIMRIK 110

 Score = 76 (38.8 bits), Expect = 0.0011, Poisson P(2) = 9.1e-12
 Identities = 12/19 (63%), Positives = 16/19 (84%)

Query:   110 QLGEMSLEEHSQCECRPKK 128
             ++GEMS  +H+ CECRPKK
Sbjct:   116 HIGEMSFLQHNKCECRPKK 134

 Score = 72 (36.8 bits), Expect = 0.0046, Poisson P(3) = 3.6e-18
 Identities = 14/21 (66%), Positives = 15/21 (71%)

Query:   202 RCQGRGLELNPDTCRCRKLRR 222
             RC +R LELN  TCRC K RR
Sbjct:   195 RCKARQLELNERTCRCDKPRR 215

 Score = 46 (23.5 bits), Expect = 47., Poisson P(4) = 7.3e-10
 Identities = 6/10 (60%), Positives = 9/10 (90%)

Query:   187 DPRTCRCRCR 196
             DP+TC+C C+
Sbjct:   181 DPQTCKCSCK 190
```

FIGURE 4

Gap Weight: 3.000 Average Match: 1.000
Length Weight: 0.100 Average Mismatch: -0.900
Quality: 100.9 Length: 739
Ratio: 0.175 Gaps: 30
Percent Similarity: 69.703 Percent Identity: 69.703

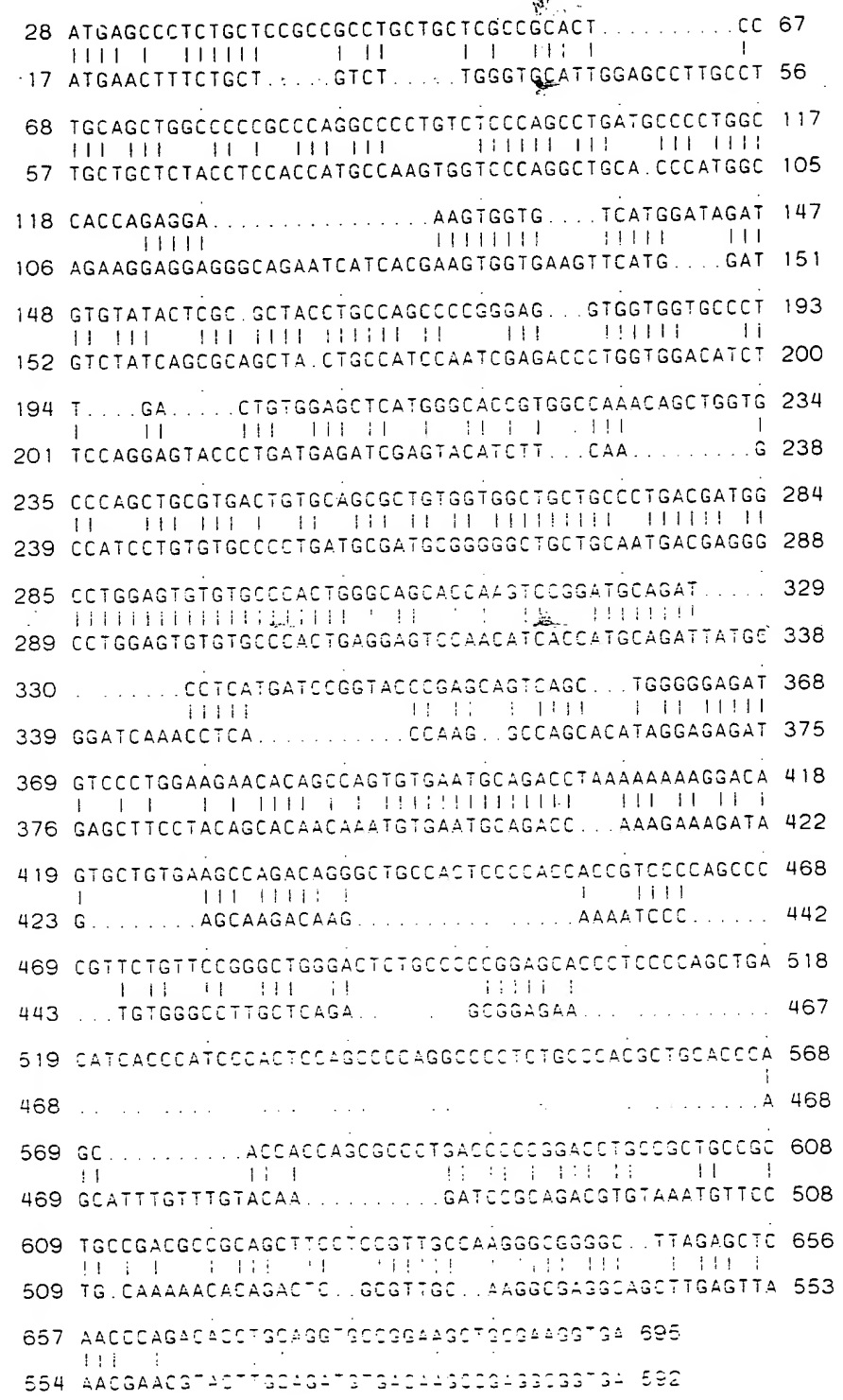


FIGURE 5A

Gap Weight: 3.000 Average Match: 0.540
Length Weight: 0.100 Average Mismatch: -0.396

Quality: 112.7 Length: 190
Ratio: 0.613 Gaps: 4
Percent Similarity: 52.459 Percent Identity: 33.333

Som175.Pep x Vegf.Pep December 21, 1994 15:55 ..

```
  1 MSPLLRRLL..LAALLQLAPA...QAPVSQPDAPGHQRKVVSWIDVYTRA 45
    |. ||.::   || || | .|   ||:. ..::..:::.||.::||| |.
  1 MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRS 50

 46 TCQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQH 95
     |:| |.:|.:  |. :.:.  : ||||.: |||||| |:||||||::
 51 YCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEES 100

 96 QVRMQILMIR.YPSSQLGEMSLEEHSQCECRPKKKDSAVKPDRAATPHHR 144
    .: |||:.|: ..: ::|||||: :|..|||||| |||.| ..:.:.:. .|
101 NITMQIMRIKPHQGQHIGEMSFLQHNKCECRP.KKDRARQENPCGPCSER 149

145 PQPRSVPGWDSAPGAPSPADITHPTPAPGPSAHAAPSTTS 184
    ... |.: :.....|  .... : ..:.:.:...
150 RKHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRCDKP 189
```

FIGURE 5B

Gap Weight: 3.000 Average Match: 0.540
Length Weight: 0.100 Average Mismatch: -0.396

Quality: 129.0 Length: 228
Ratio: 0.675 Gaps: 7
Percent Similarity: 63.243 Percent Identity: 44.324

Somnew.Pep x Vegf.Pep December 21, 1994 16:41 ..

```
  1 MSPLLRRLL..LAALLQLAPA...QAPVSQPDAPGHQRKVVSWIDVYTRA 45
    |. ||.::   || || | .|   ||:. ..::..:::.||.::||| |.
  1 MNFLLSWVHWSLALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQRS 50

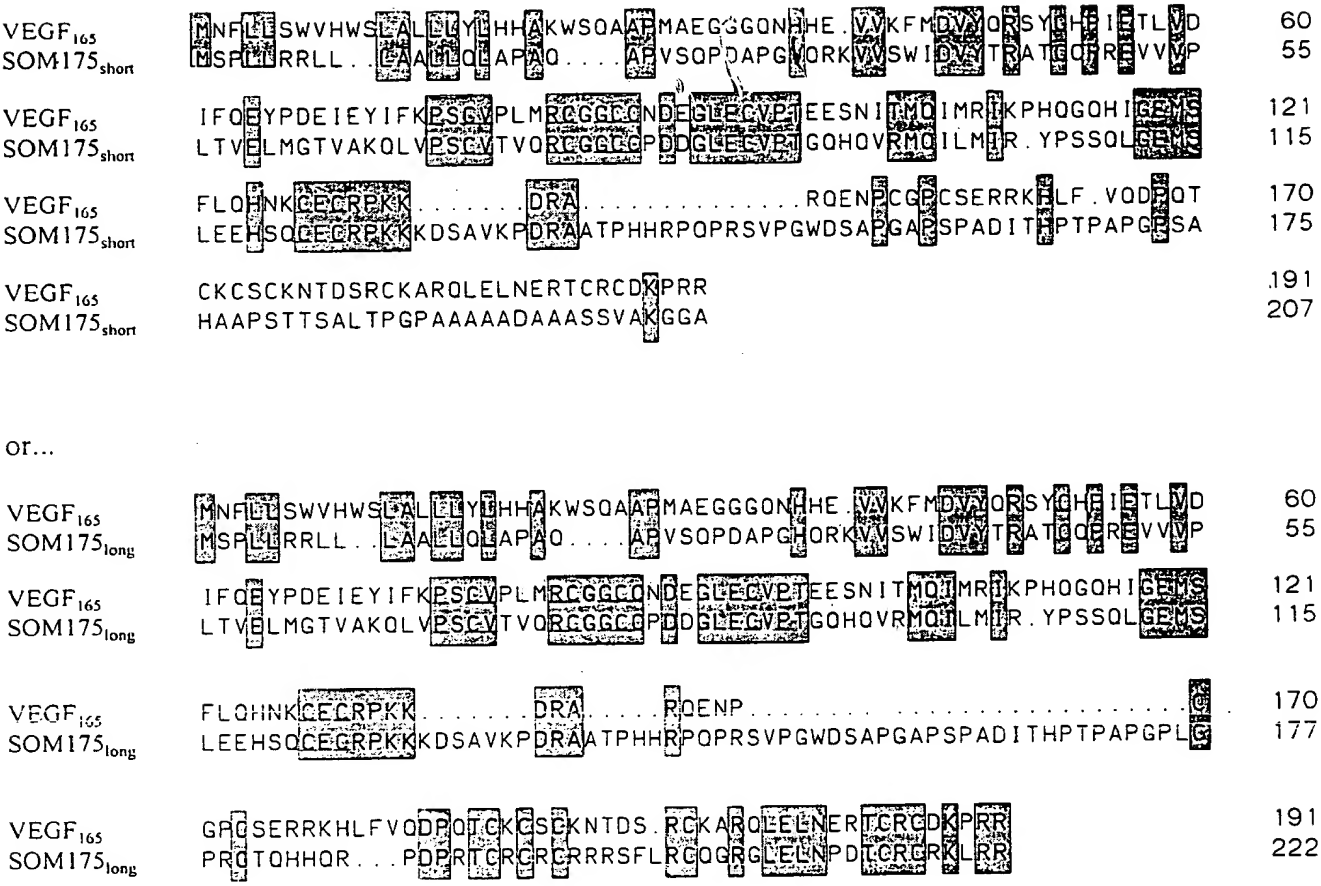
 46 TCQPREVVVPLTVELMGTVAKQLVPSCVTVQRCGGCCPDDGLECVPTGQH 95
     |:| |.:|.:  |. :.:.  : ||||.: |||||| |:||||||::
 51 YCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEES 100

 96 QVRMQILMIR.YPSSQLGEMSLEEHSQCECRPKKKDSAVKPDRAATPHHR 144
    .: |||:.|: ..: ::|||||: :|..|||||||         |||
101 NITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKK.......DRA...... 137

145 PQPRSVPGWDSAPGAPSPADITHPTPAPGPLCPRCTQHHQRPDPRTCRCR 194
          :: .|.:|:..  .|                 .||.||:|.
138 ........RQENPCGPCSERRKHLFV..............QDPQTCKCS 164

195 CRRRSFLRCCGRGLELNPDTCRCRKLRR 222
    |:.    |:.:| .|:. :'|'   .
165 CKNTDS.RCKARQLELNERTCRCDKPRR 191
```

FIGURE 6



Areas of 100% homology are boxed and conserved residues thought to be involved in homodimerisation are underlined. The VEGF sequence depicted includes the 26 amino acid leader sequence (removal of which gives rise to mature $VEGF_{165}$) giving a total length of 191 amino acids.

Homology of SOM175 to $VEGF_{165}$ is 27% (33%) at the protein level, however within this are blocks of 100% homology. In particular, many structural residues are conserved including those thought to be involved in homodimerisation of VEGF (by comparison with PDGF).

ie. Cysteine-47
Proline-70, Cysteine-72, Valine-74
Arginine-77, Cysteine-78, Glycine-80, Cysteines-81 & 82
Cysteine-89, Proline-91
Cysteines 122 & 124